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(54) Title: PHOSPHOLIPASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention provides novel polypeptides having phospholipase activity, including, e.g., phospholipase A, B, C and D activity, patatin activity, lipid acyl hydrolase (LAH) activity, nucleic acids encoding them and antibodies that bind to them. Industrial methods, e.g., oil degumming, and products comprising use of these phospholipase are also provided.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/12556

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/18, 15/00; C07H 21/04; A61K 38/46
US CL : 424/94.6; 435/197, 252.3, 320.1, 440; 536/23.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/94.6; 435/197, 252.3, 320.1, 440; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, WEST and Sequence data bases.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	Database on Genbank, Lovgren et al. 'Localization of putative virulence genes on a physical map of the Bacillus thuringiensis subsp. gelechiae choromosone.' 02 October 1998, Accession No. BTY16268. Curr. Microbiol. 37 (4), 245-250, 1998. Accession No. BTY16268 is 65.9% identical to Applicants' SEQ ID NO: 1.	1-4, 7-11, 24-26, 41-54 ---------- 5-6, 12-23, 58-60
X --- A	Database on Genbank, Lovgren et al. 'Localization of putative virulence genes on a physical map of the Bacillus thuringiensis subsp. gelechiae choromosone.' 01 June 1998, Accession No. Q52864. Curr. Microbiol. 37 (4), 245-250, 1998. Accession No. Q52864 is 82.6% identical to Applicants' SEQ ID NO: 2.	61-63, 65-70, 83-84, 88, 91-93, 108-109, 228 ---------- 64, 71-82, 85-87, 89-90, 94-101
X --- A	Database on Genbank, Gilmore et al. 'A Bacillus cereus cytolytic determinant, cereolysin AB, which comprises the phospholipase C and sphingomyelinase genes: necleotide sequence and genetic linkage.' 11 March 1996, Accession No. M24149. J. Bacteriol. 171 (2), 744-753 (1989). Accession No. M24149 is 65.5% identical to Applicants' SEQ ID NO: 1.	1-4, 7-11, 24-26, 41-54 ---------- 5-6, 12-23, 58-60

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

PCT/US03/12556

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- A	Database on Genbank, Gilmore et al. 'A Bacillus cereus cytolytic determinant, cereolysin AB, which comprises the phospholipase C and sphingomyelinase genes: necleotide sequence and genetic linkage.' 01 February 1994, Accession No. P33376. J. Bacteriol. 171 (2), 744-753 (1989). Accession No. P33376 is 82.4% identical to Applicants' SEQ ID NO: 2.	61-63, 65-70, 83-84, 88, 91-93, 108-109, 228 --- 64, 71-82, 85-87, 89-90, 94-101

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/12556

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-26, 41-54, 58-101, 108-109 & 228 [SEQ ID Nos. 1 & 2]

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1 (all in-part), claim(s) 1-26, 41-54, 58-101, 108-109 & 228, drawn to nucleic acid sequence of SEQ ID NO: 1, encoded polypeptide of SEQ ID NO: 2, vector, host cell and method of making the polypeptide recombinantly.

Groups 2-53 (all in-part), claim(s)1-26, 41-54, 58-101, 108-109 & 228, drawn to nucleic acid sequences of SEQ ID NO: 3, 5, 7, 9, ...or.105 respectively, encoded polypeptides of SEQ ID NO: 4, 6, 8, 10.....or 106 respectively, vector, host cell and method of making the polypeptide recombinantly.

[Note 1- SEQ ID NO: 3, 5, 7, 9, ...or.105 respectively refers to each of the remaining 52 nucleic acid sequences listed in claim 1, for example.
Note 2- SEQ ID NO: 4, 6, 8, 10.....or 106 respectively refers to each of the remaining 52 polypeptide sequences listed in claim 64, for example]

Group 54 (all in-part), claim(s) 27-40, drawn to nucleic acid probe comprising at least 10 consecutive bases of SEQ ID NO : 1.

Groups 55-106 (all in-part), claim(s) 27-40, drawn to nucleic acid probe comprising at least 10 consecutive bases of SEQ ID NO: 3, 5, 7, 9, ...or.105 respectively.

Group 107 (all in-part), claim(s) 55-57, drawn to antisense oligonucleotide comprising nucleic acid capable of hybridizing to the nucleic acid sequence of SEQ ID NO : 1.

Groups 108-159 (all in-part), claim(s) 55-57, drawn to antisense oligonucleotide comprising nucleic acid capable of hybridizing to the nucleic acid sequences of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively

Group 160 (all in-part), claim(s) 102-107, drawn to antibody to polypeptide sequence of SEQ ID NO : 2.

Groups 161-212 (all in-part), claim(s) 102-107, drawn to antibody to polypeptide sequence of SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 213 (all in-part), claim(s) 110-113, drawn to a method of identifying a polypeptide having phospholipase activity using the sequence of SEQ ID NO : 2.

Groups 214-265 (all in-part), claim(s) 110-113, drawn to a method of identifying a polypeptide having phospholipase activity using the sequences of SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 266 (all in-part), claim(s) 114-117, drawn to a method of identifying a a modulator phospholipase of SEQ ID NO : 2.

Groups 267-318 (all in-part), claim(s) 114-117, drawn to a method of identifying a a modulator of each of the phospholipases of SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 319, claim(s) 118-122, drawn to a computer system comprising a processor and data storage device for storing data.

Group 320, claim(s) 123-127, drawn to a method of identifying a feature in a sequence or for comparing sequences using a computer program.

Group 321 (all in-part), claim(s) 128-132, drawn to a method of recovering a nucleic acid encoding the polypeptide of SEQ ID NO: 2.

Groups 322-373 (all in-part), claim(s) 128-132, drawn to a method of recovering a nucleic acid encoding each of the polypeptide sequence of SEQ ID NO: 4, 6, 8, 10.....or 106 respectively.

Group 374 (all in-part), claim(s) 133-147, drawn to a method of generating a variant of nucleic acid sequence of SEQ ID NO : 1.

Group 375-426 (all in-part), claim(s) 133-147, drawn to a method of generating a variant of each of the nucleic acid sequence of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively.

Group 427 (all in-part), claim(s) 148-157, drawn to a method of producing a library encoding a plurality of modified phospholipase derived from nucleic acid sequence of SEQ ID NO : 1.

Group 428-479 (all in-part), claim(s) 148-157, drawn to a method of producing a library encoding a plurality of modified phospholipase derived from nucleic acid sequence of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively.

Group 480 (all in-part), claim(s) 158-159, drawn to a method of determining a functional fragment of a phospholipase, wherein the enzyme comprises the sequence of SEQ ID NO : 2.

Groups 480-532 (all in-part), claim(s) 158-159, drawn to a method of determining a functional fragment of a phospholipase, wherein the enzyme comprises the sequence of SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 533 (all in-part), claim(s) 160-163, drawn to a method for whole cell engineering, method comprising making a modified cell by modifying the nucleic acid sequence of SEQ ID NO : 1.

Group 534-585 (all in-part), claim(s) 160-163, drawn to a method for whole cell engineering, method comprising making a modified cell by modifying the nucleic acid sequence of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively.

Group 586 (all in-part), claim(s) 164-169, drawn to an isolated signal sequence comprising residues 1-16 to 1-33 of SEQ ID NO : 2.

Group 587-638 (all in-part), claim(s) 164-169, drawn to an isolated signal sequence comprising residues 1-16 to 1-33 of SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 639 (all in-part), claim(s) 170, drawn to a method of over-expression using nucleic acid sequence of SEQ ID NO : 1.

Group 640-691 (all in-part), claim(s) 170, drawn to a method of over-expression using nucleic acid sequence of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively.

Group 692 (all in-part), claim(s) 171-174, drawn to a method of making a transgenic plant by introducing SEQ ID NO: 1 into a cell.

Groups 693-744 (all in-part), claim(s) 171-174, drawn to a method of making a transgenic plant by introducing SEQ ID NO: 3, 5, 7, 9, ...or.105 respectively, into a cell.

Group 745 (all in-part), claim(s) 175-199, drawn to a method of hydrolyzing phospholipid using a polypeptide encoded by the nucleic acid sequence of SEQ ID NO: 1.

Group 745-797 (all in-part), claim(s) 175-199, drawn to a method of hydrolyzing phospholipid using a polypeptide encoded by the nucleic acid sequence of SEQ ID NO: 3, 5, 7, 9, ...or.105 respectively.

Group 798 (all in-part), claim(s) 200-204, drawn to a method of caustic refining of phospholipid composition using SEQ ID NO : 2.

Group 799-850 (all in-part), claim(s) 200-204, drawn to a method of caustic refining of phospholipid composition using SEQ ID NO : 4, 6, 8, 10.....or 106 respectively.

Group 851 (all in-part), claim(s) 205-227, drawn to a method for purification of phytosterol or triterpene using polypeptide encoded by the nucleic acid sequence of SEQ ID NO : 1.

Groups 852-903 (all in-part), claim(s) 205-227, drawn to a method for purification of phytosterol or triterpene using polypeptide encoded by the nucleic acid sequence of SEQ ID NO : 3, 5, 7, 9, ...or.105 respectively. The inventions listed as Groups 1-903 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Group I has a special technical feature of a nucleotide sequence

of SEQ ID NO : 1, encoding the polypeptide sequence of SEQ ID NO : 2, host cell, and a method of making the protein, which Groups 2-903 do not share; Groups 2-53, each has a distinct nucleic acid and polypeptide sequence, which Groups 1 & 54-903 do not share; Groups 54-106, each has a special technical feature of a distinct nucleic acid probe to a fragment each of the nucleic acid sequence of SEQ ID NO : 1, 3, 5, 7,.... or 105, respectively; which groups 1-53 & 107-903 do not share; Groups 107-159, each has a special technical feature of a distinct antisense oligonucleotide to each of the nucleic acid sequence of SEQ ID NO : 1, 3, 5, 7,....or 105, respectively; which groups 1-106 & 160-903 do not share; Groups 160-212, each has a special technical feature of a distinct antibody to each of the polypeptides of SEQ ID NO : 2, 4, 6, ... or 106, respectively; which groups 1-159 & 213-903 do not share; Groups 586-638, each has a special technical feature of a distinct signal sequence to each of the polypeptide sequence of SEQ ID NO : 2, 4, 6, ...106, respectively; which groups 1-585 & 639-903 do not share; Groups 213-585 & 639-903 employ distinct nucleic acid sequences of SEQ ID Nos. 1, 3, 5, ..or 105 or distinct polypeptide sequence of SEQ ID Nos. 2, 4, 6, ... or 106, respectively, however, in view of 37 CFR 1.475 (b), when claims corresponding to different categories of inventions are present then only (3) applies and additional methods of use are deemed to lack unity. Thus the various groups discussed above show a lack of unity of invention.